REMARKS

This paper is filed in response to the final official action dated November 29, 2007 (hereafter, the "official action"). This paper is timely-filed.

Claims 1-2, 4-5, and 7-13 are pending. All pending claims 1-2, 4-5, and 7-13 have been rejected under 35 U.S.C. §103(a) as assertedly obvious over Saito *et al.*, *J. Cerebral Blood Flow Metabol., 17:*857-864 (1997) ("Saito") in view of Gray *et al.*, GB 2350297 ("Gray"). The applicants respectfully traverse the rejections.

Saito discloses administering to cats, via inhalation, 0.75% halothane in 70% nitrous oxide and 30% oxygen, which corresponds to an amount of halothane sufficient to maintain a general anesthetic effect. In support of applicant's assertion that the dose is sufficient to maintain a general anesthetic effect in cats, applicants enclose herewith Toyota *et al.*, *Stroke, 33:*1383-1391 (2002),[1] entitled "Malignant Infarction in Cats After Prolonged Middle Cerebral Artery Occlusion," which discloses that a general anesthetic effect can be maintained by administering to cats, via inhalation, as little as 0.6% halothane in a 70% nitrous oxide/30% oxygen gas mixture.

In marked contrast, all pending claims recite a method of treating a patient having a tissue that is subject to an ischemic event comprising *parenterally administering* a formulation comprising a halogenated volatile anesthetic to the patient *in a sub-anesthetic amount* effective to improve the tissue's resistance to or tolerance of the ischemic event.

Saito neither discloses nor suggests such a method. Rather, as previously demonstrated, Saito only discloses administering an *anesthetic amount* of halothane *via inhalation*. Furthermore, Saito explicitly indicates that any ischemic protective effect demonstrated therein is limited to administering an *anesthetic amount* of halothane by distinguishing between the awake and anesthetized states:

> *Compared with the awake state*, volatile anesthetics, including halothane and sevoflurane, reduce brain damage in animals subjected to transient focal cerebral ischemia.

See Saito at page 862 (emphasis added). Therefore, one cannot separate the protective effect demonstrated in Saito from the induced anesthetic state, as proposed by the examiner.

Furthermore, the examiner's indication at pages 2-3 of the action that Saito suggests that organs other than brain tissues would be protected from ischemic events by

[1] The document is attached hereto as Attachment A.

administration of halothane is unsupported by any factual basis. The applicants respectfully submit that it is the examiner's burden of proof to demonstrate a factual basis for the aforementioned assertion.[2] Moreover, the applicants respectfully submit that it is well known that the target organ of general anesthetic agents like halothane is the brain. One of ordinary skill would therefore not expect that a protective effect would be demonstrated in non-target tissues based on an effect that was shown in target tissues. Thus, at least claim 13 is patentable over the cited art for this additional reason.

Finally, the proposed combination of Saito with Gray is flawed. Gray discloses adapting halogenated volatile anesthetics for injectable administration so as to be capable of *inducing* an anesthetic effect. Saito merely discloses using halothane – a halogenated volatile anesthetic – as an agent for maintaining a general anesthetic effect. Accordingly, one of ordinary skill would not look to Gray to modify the composition used in the method disclosed by Saito.

For at least the foregoing reasons, a *prima facie* case of obviousness cannot be sustained.

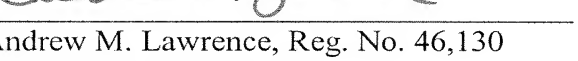
CONCLUSION

It is respectfully submitted that this application is now in condition for allowance. Should the examiner wish to discuss the foregoing, or any matter of form or procedure in an effort to advance this application to allowance, he is respectfully invited to contact the undersigned attorney at the indicated telephone number.

Respectfully submitted,

MARSHALL, GERSTEIN & BORUN LLP

May 29, 2008



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[2] *See In re Piasecki*, 745 F.2d 1468, 1472, (Fed. Cir. 1984): "The Supreme Court in *Graham v. John Deere Co.*, 383 U.S. 1, 86 S. Ct. 684, 15 L. Ed. 2d 545, 148 U.S.P.Q. 459, (1966), focused on the procedural and evidentiary processes in reaching a conclusion under section 103. As adapted to ex parte procedure, Graham is interpreted as continuing to place the "burden of proof on the Patent Office which requires it to produce the factual basis for its rejection of an application under sections 102 and 103". *In re Warner*, 379 F.2d 1011, 1016, 154 U.S.P.Q. 173, 177 (CCPA 1967).

"Attachment A"

Malignant Infarction in Cats After Prolonged Middle Cerebral Artery Occlusion

Glutamate Elevation Related to Decrease of Cerebral Perfusion Pressure

Shingo Toyota, MD; Rudolf Graf, PhD; Mario Valentino, PhD; Toshiki Yoshimine, MD; Wolf-Dieter Heiss, MD

Background and Purpose—To study the putative role and predictive significance of glutamate elevation in space-occupying ischemic stroke, we investigated the correlation between perfusional disturbances and glutamate alterations in a transient ischemia model in cats that is susceptible to secondary deterioration after reperfusion.

Methods—In 10 halothane-anesthetized cats, the left middle cerebral artery was occluded for 3 hours, followed by 6 hours of reperfusion. Laser-Doppler flowmetry (LDF) probes, microdialysis/high-performance liquid chromatography, and pressure sensors measured simultaneously regional cerebral blood flow (CBF), extracellular amino acids, mean arterial blood pressure, and intracranial pressure, respectively. Cerebral perfusion pressure (CPP) was calculated. In complementary experiments (n=2), regional CBF was assessed by sequential positron emission tomography.

Results—Middle cerebral artery occlusion reduced LDF-measured CBF in all animals to <25% of control. In 5 of 10 cats, glutamate rose approximately 30-fold during ischemia. LDF-measured CBF and glutamate primarily recovered after reperfusion. Glutamate rose again in the late reperfusion phase, when CPP decreased to <60 mm Hg, and symptoms of transtentorial herniation were recognized. Positron emission tomography revealed ischemic thresholds of 15 to 20 mL/100 g per minute for secondary deterioration. In the other 5 cats, ischemic elevation of glutamate was significantly smaller, and signs of secondary deterioration were not recognized.

Conclusions—Glutamate determinations during ischemia predict fatal outcome, as do intracranial pressure and CPP measurements during early reperfusion. Secondary amino acid elevation during reperfusion is presumably caused by a drastic decrease of CPP to <50 mm Hg in the final stage of space-occupying, malignant focal ischemia. At this stage, a further progression of injury due to increased glutamate may be irrelevant with respect to fatal outcome. (***Stroke*. 2002; 33:1383-1391.)**

Key Words: cerebral infarction ■ cerebral ischemia, focal ■ glutamates ■ intracranial pressure ■ perfusion ■ tomography, emission computed ■ cats

In 2 recent clinical trials, early thrombolytic therapy of acute ischemic stroke has been shown to be effective if a certain number of inclusion criteria are met.[1–6] Risks of reperfusion therapy, however, are still under debate,[7] and among these, malignant, space-occupying brain edema may be one important factor that narrows the therapeutic window. In experimental studies, reperfusion may exacerbate tissue injury if initiated beyond the time window for viability of brain tissue.[8,9] A mechanism by which this type of injury is accomplished is intracranial hypertension provoked by excessive, and in certain instances malignant, vasogenic edema[10,11] and by resulting transtentorial herniation.[12]

Excitatory amino acids, especially L-glutamate (Glu), have been considered to play a key role in the course of anoxic and ischemic brain damage.[13–15] With intracerebral microdialysis, excessive release of Glu into extracellular space has been documented in various animal models of experimental stroke.[16–20] It has been shown that the volume of damage in an ischemic focus correlates with the amount of Glu release.[17] Only recently has secondary elevation of amino acids after prolonged transient ischemia been reported [21–23]. It has been suggested that perturbation in release-reuptake mechanisms of amino acids and secondary deterioration of cerebral blood flow (CBF) may account for this secondary increase, but the mechanisms in relation to edema formation remain obscure. In the search for monitoring tools that permit early identification of such fatal outcome, microdialysis has recently been applied in the care of patients with severe stroke.[24,25]

In the present study we investigated a model of transient focal ischemia in cats that has been shown to be prone to

Received December 10, 2001; final revision received January 14, 2002; accepted February 5, 2002.

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Stroke is available at http://www.strokeaha.org

DOI: 10.1161/01.STR.0000015557.18508.DD

secondary deterioration during reperfusion if the duration of the ischemic episode is prolonged to 2 to 4 hours.[21] By choosing a 3-hour ischemic period followed by reperfusion, we expected that a significant number of experiments would lead to transtentorial herniation, and we hypothesized that glutamate elevation is involved in this process. With the use of a multiparametric approach, the study focused on time course relationships between intracranial pressure (ICP) and cerebral perfusion pressure (CPP), CBF, and extracellular alterations of neurochemical substances to investigate mechanisms of primary and secondary glutamate elevation in relation to a malignant course of edema formation and global CBF reduction. In complementary experiments, the time course of perfusional alterations in the whole brain was assessed by positron emission tomography (PET).

Materials and Methods

Animal Preparation

Ten cats weighing 3.2 to 4.7 kg were used. The study was approved by the local Animal Care Committee and the Regierungspräsident of Cologne and is in compliance with the German Laws for Animal Protection. General anesthesia was initiated by ketamine hydrochloride (25 mg/kg IM). The left femoral artery and vein were cannulated to administer drugs and to measure mean arterial blood pressure (MABP) and arterial blood gases. The animals were tracheotomized and immobilized with pancuronium bromide (0.2 mg/kg IV). Thereafter, artificial ventilation was initiated, and anesthesia was changed to halothane (0.6% to 1.2%) in a 70% nitrous oxide/30% oxygen gas mixture. Intravenous infusion of Ringer's solution containing 5 mg/kg per hour gallamine triethiodide for muscle relaxation was maintained throughout the experiment. Arterial blood gases were measured intermittently. Deep body temperature was kept at 37.0°C with the use of a heating blanket that was feedback controlled by a rectal temperature probe.

The left middle cerebral artery (MCA) was exposed transorbitally; an occlusion device was implanted at the proximal portion of the MCA and completely fixed with rapid-drying glue. The orbita was sealed with dental cement to prevent leakage of cerebrospinal fluid (CSF). Small burr holes were drilled in the skull above a region in the ischemic core (left ectosylvian gyrus) and above a perifocal region of the ischemia (left marginal gyrus). In both sites, the dura was removed under microscopic control, and microdialysis probes (see below) were inserted into gray matter of ectosylvian gyrus (n=10) and marginal gyrus (n=9). The depth of insertion was adjusted to 1.5 mm. Adjacent to the microdialysis probes, laser-Doppler flowmetry (LDF) probes (tip diameter, 0.8 mm; Moor Instruments) for measurement of regional CBF were placed on the cortical surface with the use of a micromanipulator. A strain-gauge MicroSensor probe for measurement of ICP (Codman/Johnson & Johnson Professional, Inc) and a thermocouple for measurement of regional brain temperature were additionally placed on the cortical surface adjacent to the microdialysis probe at the site above the ectosylvian gyrus. The burr holes were completely sealed with dental cement to prevent CSF leakage. Brain temperature was kept at 37.0°C with the use of a feedback-controlled heating lamp system.

Microdialysis

Microdialysis probes were manufactured as concentric tubes with an inner and outer silica tube and a capillary dialysis membrane at the tip glued to the outer tube (Cuprophan, Akzo Nobel; cutoff, 6000 Da; diameter, 250 μm; length of active membrane, 1.5 mm). The probes were continuously perfused with artificial CSF (in mmol/L: $CaCl_2$ 1.2, NaCl 145, KCl 2.7, $MgCl_2$ 1.0, adjusted to pH 7.4 with phosphate buffer) at a flow rate of 2 μL/min. At this flow rate, in vitro recovery of glutamate for the probes used in the study amounted to 13.1±3.2%. Microdialysis probes were perfused continuously with artificial CSF at a constant flow rate of 2.0 μL/min with the use of a microinfusion pump (CMA/100; Carnegie Medicine). Amino acids were analyzed by high-performance liquid chromatography with the use of an RF-535 fluorescence detector (excitation wavelength, 330 nm; emission wavelength, 480 nm; Shimadzu) after separation by a 5-μL C18-Nucleosil column (60×4.0 mm; Knauer) with the use of a gradient elution profile (buffer A: 0.1 mol/L sodium acetate, pH 5.7; buffer B: 100% methanol) after precolumn derivatization with *o*-phthaldialdehyde.[26]

Positron Emission Tomography

In complementary experiments (n=2), the course of regional changes of CBF was assessed for the whole brain by PET. The experiments were performed in a clinical high-resolution PET camera (Siemens/CTI ECAT EXACT HR). The method has been described elsewhere.[27] In brief, the scanner has a field of view of 15 cm, an in-plane spatial resolution of 3.6 mm full width at half maximum, and an axial resolution of 4.0 mm full width at half maximum.[28] The animal was positioned and kept in the scanner gantry throughout the entire experiment to guarantee positional stability. CBF was determined after intravenous bolus injection of 20 mCi ^{15}O-labeled water. Experimental background and limitations of this method for measurement of cerebral hemodynamics have been previously discussed.[29] The cats used in the PET experiment were otherwise treated in the same way as the rest of the animals included in the study.

Experimental Protocol

The variables (ICP, CBF measured by LDF, MABP, brain temperature, end-tidal CO_2) were continuously recorded with the use of a PC-based data acquisition system (DASY LAB). After a stabilization period of at least 2 hours, control microdialysis samples were collected, and control measurements of the various parameters were taken. Thereafter, the MCA was occluded for 3 hours and then reopened. The observation period was followed 6 hours into the reperfusion phase.

Histology

The experiments were terminated by perfusion fixation with 4% paraformaldehyde solution if the animals did not die in the course of malignant infarction. Brains were removed and additionally immersion fixed for at least 2 weeks. After paraffin embedding, 7-μm-thick coronal sections of the brain were cut at distances of 2 mm and stained with hematoxylin-eosin or with a combination of Luxol fast blue and cresyl violet. Brain damage was macroscopically and microscopically determined on individual brain sections.

Statistical Analysis

All data are expressed as mean±SD. The significance of differences at $P<0.05$ was tested between sequential measurements or between groups by ANOVA and multiple post hoc comparisons (Fisher's protected least significant difference) (Statistica, StatSoft Inc).

Results

In individual cats of this experimental series, 3-hour MCA occlusion (MCAO) and subsequent reperfusion resulted in 2 distinct but different types of courses of the various parameters that were measured by invasive techniques. In 1 group of cats, a malignant course was observed with secondary deterioration after primary recovery of the parameters that had been disturbed during the ischemic episode. In another group, a benign course was apparent with continued recovery of the various parameters. Details of the grouping criteria are explained below with the help of actual multiparametric recordings. Thereafter, the results of this series are given, followed by the presentation of an experiment with malignant course that was performed in a high-resolution PET scanner. With the use of sequential imaging of regional CBF by PET,

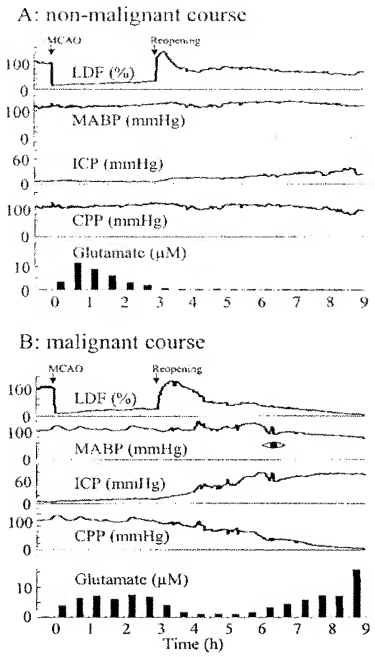

Figure 1. Plots of actual recordings obtained in ectosylvian gyri of 2 animals exhibiting a nonmalignant (A) and a malignant (B) course of infarction. In the malignant course (B), note secondary rise of ICP and drop of CPP after reperfusion preceding right pupillary dilation (marked by an eye symbol) and secondary rise of glutamate. Glutamate refers to concentration of glutamate in dialysate.

regional transitions from control into the ischemic state, and thereafter into postischemic hyperperfusion and hypoperfusion and finally into global ischemia, can be documented for the whole brain.

Actual Recordings

Figure 1 shows actual recordings of 2 experiments (A and B) that differed in outcome. In both instances, MCAO primarily reduced LDF-measured CBF in the ischemic core to <10% to 20% of control. In both experiments, CBF measured by LDF spontaneously increased thereafter to some extent. During the ischemic episode, MABP, ICP, and CPP did not show major changes, and extracellular glutamate rose in the 2 experiments to almost the same level of approximately 10 μmol/L. In experiment A, however, glutamate started to decrease during the ischemic episode, whereas it remained high in experiment B. Differences between the 2 experiments become more apparent during the reperfusion phase.

Recirculation primarily increased LDF-measured CBF in both instances to or even above control levels. This phenomenon is typically referred to as postischemic hyperperfusion. After this transient rise, LDF-measured CBF fell below control to a level that may be referred to as postischemic hypoperfusion. In experiment A, LDF-measured CBF remained on the level of hypoperfusion throughout the observation period, whereas in experiment B, it stayed on this level only for a brief period. It continued to decrease thereafter, starting at approximately 3 hours after reperfusion, and ceased in the final stage.

Physiological Variables in Cats Subjected to MCAO

	pH	Pco_2, mm Hg	Po_2, mm Hg
Group with malignant course			
Control before MCAO	7.38±0.03	31.28±1.51	147.20±14.43
1 h after MCAO	7.35±0.01	32.00±1.77	141.78±10.81
5 h after MCAO	7.36±0.02	30.88±2.46	139.80±8.68
Group with nonmalignant course			
Control before MCAO	7.36±0.01	31.97±1.16	147.63±21.42
1 h after MCAO	7.35±0.02	32.10±2.11	135.48±18.08
5 h after MCAO	7.34±0.02	32.40±1.76	139.03±16.82

Values are mean±SD; n=5 in both groups.

MABP did not show major changes in experiment A, whereas in experiment B, it increased somewhat, reaching approximately 150 mm Hg at 3 hours after recirculation. Thereafter, it suddenly dropped to <100 mm Hg. This event may correspond to a medullary stage of herniation that has been described as Cushing's phenomenon. It was preceded in this experiment by eye dilatation.

ICP increased only slightly in experiment A, but it continued to increase in experiment B, peaking at approximately 80 mm Hg. After reaching this peak, ICP decreased briefly, concomitantly with the described drop of MABP, and continued to increase thereafter.

Corresponding to the changes in MABP and ICP, CPP remained almost normal in experiment A, whereas in experiment B, it continuously decreased and showed a sudden additional drop exactly at the time when MABP decreased to <100 mm Hg. CPP finally fell to approximately 10 mm Hg.

With regard to alterations of extracellular glutamate, ischemic levels returned to preischemic control values in experiment A. In experiment B, however, a secondary elevation was observed in the late reperfusion phase, finally reaching a concentration of approximately 17 μmol/L. This increase coincided with a CPP decrease to <50 to 60 mm Hg. Since secondary glutamate elevation proved to be in all experiments the clearest denominator for a malignant course, we took this phenomenon as a grouping criterion for further analysis of the experiments. Therefore, the 2 groups were termed the malignant group (n=5) and the nonmalignant group (n=5), respectively.

Blood Gases

In both groups, physiological variables, including arterial blood Pao_2, $Paco_2$, and pH, could be kept within normal range throughout almost the entire experimental protocol (Table). In the malignant group, during the final stages after herniation, blood samples were not obtained to avoid disturbances of MABP. In single cases, however, it was possible to document that during this stage, autoregulation is lost. In particular, $Paco_2$ rises, and pH exhibits a trend toward acidic ranges.

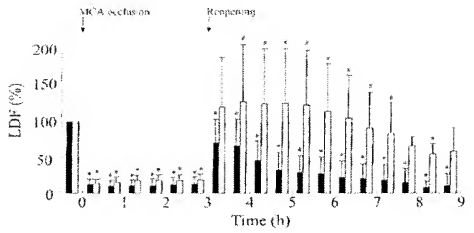

Figure 2. Changes in LDF in the 2 groups exhibiting a nonmalignant (white bars; n=5) and a malignant (black bars; n=5) course of infarction as a function of time. Mean±SD values are plotted every half hour. *$P<0.05$, significantly different from preischemic control; #$P<0.05$, significantly different from group exhibiting a malignant course.

CBF Measured by LDF

MCAO reduced LDF-measured CBF in the ischemic core in all animals to <25% of control (Figure 2). During ischemia, differences between the 2 groups were negligible. After reperfusion, recovery of LDF-measured CBF was complete, with some indication of postischemic hyperperfusion only in the nonmalignant group. In the malignant group, reperfusion increased LDF-measured CBF to only approximately 70% in the earlier stage; this value later decreased continuously until the end of the observation period. LDF-measured CBF values of the malignant group were significantly lower than those of the nonmalignant group for most of the time after reperfusion.

MABP, ICP, and CPP

MABP did not change throughout MCAO (Figure 3). After reperfusion, it increased slightly in the nonmalignant group, whereas in the malignant group, it significantly decreased in the later phase of the experiment at approximately 7 to 8 hours after MCAO, reaching a level of <100 mm Hg. In the late phase, MABP was significantly lower in the malignant than in the nonmalignant group.

Similar to MABP, ICP did not change during MCAO; however, it started to increase in both groups almost immediately after reopening of the MCA (Figure 3). In the early phase of reperfusion, this increase was much more pronounced in the malignant group. It continued to rise in this group throughout the observation period and reached final values of >70 mm Hg. In contrast, in the nonmalignant group, MABP remained at the marginally elevated level that had already been reached in the early reperfusion phase.

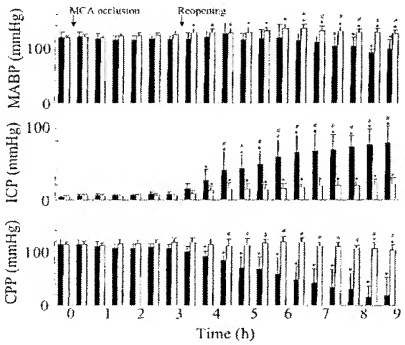

Figure 3. Changes in MABP, ICP, and CPP in the 2 groups exhibiting a nonmalignant (white bars; n=5) and a malignant (black bars; n=5) course of infarction as a function of time. Mean±SD values are plotted every half hour. *$P<0.05$, significantly different from preischemic control; #$P<0.05$, significantly different from group exhibiting a malignant course.

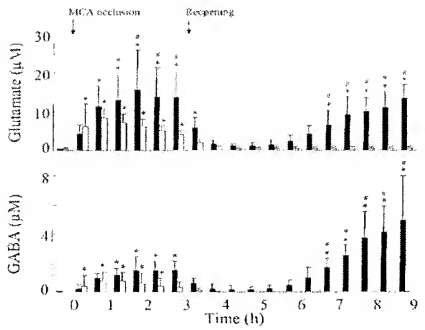

Figure 4. Changes in glutamate and GABA in the 2 groups exhibiting a nonmalignant (white bars; n=5) and a malignant (black bars; n=5) course of infarction as a function of time. Mean±SD values are plotted every half hour. *$P<0.05$, significantly different from preischemic control; #$P<0.05$, significantly different from group exhibiting a malignant course.

As a consequence of MABP and ICP alterations, CPP did not change during the occlusion phase, and in the nonmalignant group, it remained almost unaltered throughout reperfusion (Figure 3). In the malignant group, in contrast, CPP started to fall immediately after reopening of the MCA, and this decrease continued until values of <30 mm Hg were reached, indicating severe global disturbance of cerebral perfusion.

Glutamate and γ-Aminobutyric Acid

MCAO increased extracellular glutamate and γ-aminobutyric acid (GABA) in both the nonmalignant and the malignant groups (Figure 4). The magnitude of this elevation, however, was much larger in those animals that developed a malignant course later after reperfusion, and in this group, the peak of substrate elevation appeared later (at approximately 2 hours after occlusion) than in the group with a nonmalignant course (at approximately 1 hour after occlusion). After reperfusion, both glutamate and GABA levels recovered quickly within almost 1 hour. Dramatic secondary elevations of both extra-

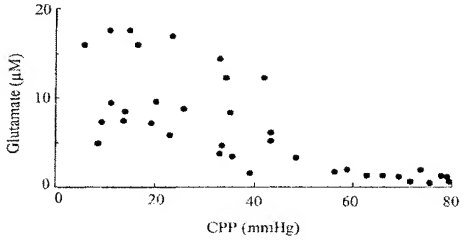

Figure 5. Relation between CPP and glutamate elevation in the group exhibiting a malignant course of infarction. Data were obtained from the phase after reperfusion that followed the primary recovery (see Figures 3 and 4). Thirty-minute data were plotted. Note the LDF threshold for extracellular glutamate elevation at a CPP of 40 to 50 mm Hg.

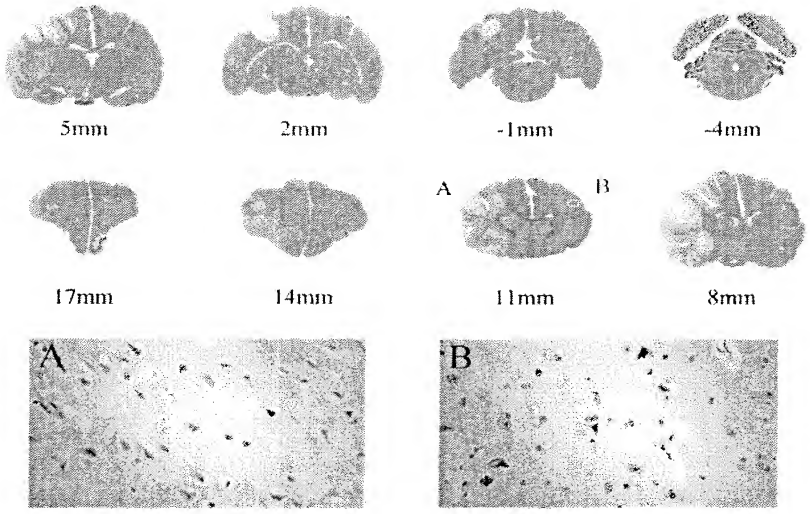

Figure 6. Cross sections (stained with hematoxylin-eosin) of an animal of the group exhibiting a malignant course of infarction (top). Infarctions cover large parts of the MCA territory. Note massive midline shifts documenting excessive brain swelling. Histological analysis revealed that early neuronal necrosis was apparent in the cortex both ipsilateral (A) and contralateral (B) to the occluded MCA.

cellular substrates were observed in the malignant group approximately 6 to 7 hours after MCAO. At this time, differences between groups again became significant. GABA in particular continued to rise and reached final levels that were much higher than those observed during MCAO. In the nonmalignant group, in contrast, no such secondary increase was observed.

Relationship Between CPP and Glutamate

To demonstrate the dependency of extracellular glutamate levels after recirculation on CPP alterations in the malignant group, data derived from the phase that followed the primary recovery of extracellular substrate levels after reperfusion were plotted against each other (Figure 5). A threshold for secondary glutamate elevation became apparent at CPP levels of approximately 40 to 50 mm Hg.

Histology

In brains of the malignant group, histological analysis revealed infarctions covering large parts of the MCA territory (Figure 6, top). Massive midline shifts were always apparent, documenting excessive brain swelling. In this group, early neuronal necrosis (neuronal perikarya with shrinkage and triangulation of the nucleus and cytoplasm and increased basophilia of the cytoplasm[30–32]) was recognized not only in the cortex ipsilateral to the occluded MCA (Figure 6A) but also in the contralateral cortex (Figure 6B). Furthermore, hemorrhagic infarction was not apparent in any of the investigated brains.

PET: Regional CBF Changes During Malignant Course

Sequential PET measurements of CBF revealed that in case of a malignant course, MCAO induced ischemia in a large focus in the left hemisphere (Figure 7; the black line demarcates an isocontour of CBF <20 mL/100 g per minute during the ischemic phase). Immediately on reperfusion, a large portion of the focus was hyperperfused and turned into hypoperfusion 1 to 2 hours later. In the further course of the experiment, CBF decreased progressively in the formerly ischemic hemisphere. At a final stage presumably characterized by transtentorial herniation, CPP fell to <50 to 60 mm Hg, and CBF reduction spread into the contralateral hemisphere, resulting in global ischemia.

Discussion

This study was designed to investigate the temporal relationship between multiple parameters involved in the progression of malignant edema formation after prolonged ischemia and reperfusion. The intention was not only to investigate the pathophysiological sequelae that led to a malignant course but also to identify those parameters that predict this course in an early stage.

An important prerequisite for this approach was that in the current focal ischemia model in cats, the probability of induction of a malignant course was in the 50% range, so that a "malignant group" could be directly compared with the rest of the animals exhibiting a benign course. Heterogeneity in outcome among individual cats may derive from several factors, including variations in the vascular supply of the brain and in the degree of collateralization as well as differences in basic metabolic or functional conditions of the particular animal. This heterogeneity is well known from work in ischemia models in larger animals[33,34] and may reflect, to a certain extent, variations in stroke patient outcome, particularly after thrombolysis.[1,3]

Secondary or delayed tissue injury following primary recovery after reperfusion is a common feature that has been described many times in experimental stroke. It seems to depend mainly on the duration of the ischemic epoch and on the severity of the ischemic insult. Brief ischemic episodes of less than approximately 30 minutes are normally characterized by full and persistent recovery, whereas intermediate ischemic episodes of 30 to 60 minutes may result in secondary damage. This damage, usually referred to as reperfusion injury, is associated with a variety of mechanisms that appear during the reperfusion phase. Such mechanisms include free radical formation,[35] protein synthesis inhibition,[36] and apo-

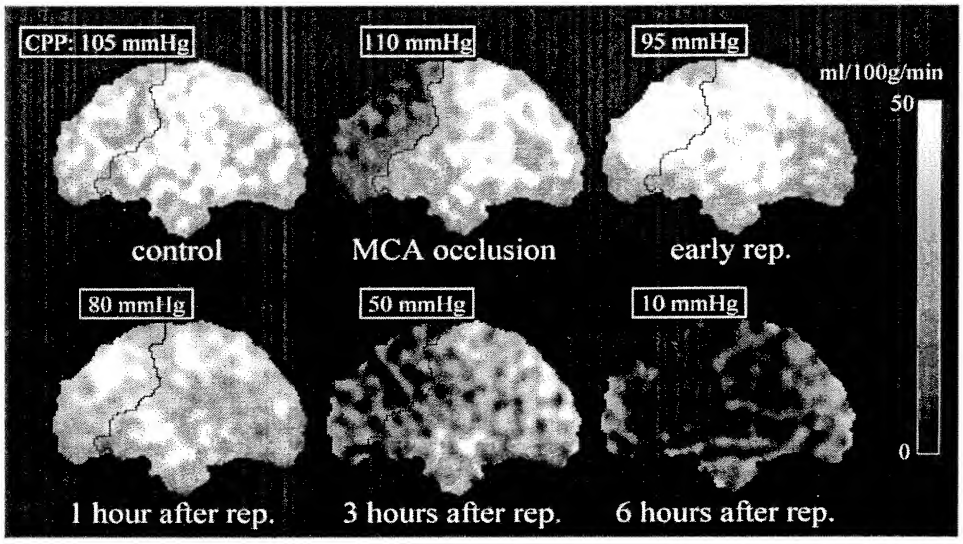

Figure 7. Sequential PET images of CBF obtained in 1 coronal plane of an individual cat exhibiting a malignant course of infarction. Note the decrease of CBF in the MCA territory during occlusion (demarcated by black line) and the subsequent hyperperfusion in the center of the formerly ischemic territory immediately after reperfusion (early rep.). Note also that in the last 3 images of the sequence (bottom), CBF drops again, starting in the MCA territory and then, at a CPP of 50 mm Hg, proceeding to other brain regions, including the hemisphere contralateral to the occluded MCA.

ptosis.[37] Whether excitotoxicity plays a role in this type of damage remains obscure. Indications for such a role may derive from studies that show secondary elevation of glutamate and associated amino acids, such as aspartate, during the reperfusion phase.[21–23] Another type of secondary damage presumably underlying secondary deterioration that has been observed in the present study is caused by blood-brain barrier disruption and associated vasogenic edema formation. This type is only induced after prolonged ischemic episodes of 2 to 4 hours[8,38] and leads to subsequent rise of ICP as a result of brain swelling.[11,39] Edema formation seems to be a major risk of recanalization after thrombolysis with recombinant tissue plasminogen activator in stroke patients.[40,41] Evidence exists that space-occupying edema does not occur more often in patients undergoing thrombolysis than in normal patients, but it may be aggravated by the thrombolytic treatment.[7] Interestingly, in a recent nuclear MR study comparing 30 minutes, 60 minutes, and 2.5 hours of transient MCAO in rats, apparent lesion volumes calculated from diffusion- and T2-weighted images revealed no significant differences 7 days after reperfusion. Blood-brain barrier damage assessed by enhancement on postcontrast T1-weighted images, however, and the mortality rate were increased in the group with 2.5 hours of occlusion.[42] Therefore, the most relevant difference between intermediate and prolonged transient ischemia seems to be the appearance of a malignant course of edema formation that putatively results in drastic brain swelling and perhaps transtentorial herniation, including transition to global brain ischemia and death.

In the cat model that was used in the present study, 2 hours of transient MCAO have been shown to result in blood-brain barrier breakdown and in significant water accumulation in the ischemic focus.[9] Taguchi et al[21] showed in the same model that 4-hour occlusion regularly resulted in secondary glutamate elevation measured 15 hours after reperfusion. Thus, 3 hours of MCAO with 6 hours of subsequent reperfusion seemed appropriate, and it was possible to observe the full course of edema formation and rise in ICP up to the point of transtentorial herniation and subsequent transition to global ischemia. Additionally, this course occurred in a reasonable time frame of approximately 10 hours, which permitted sequential PET scans with the resultant 3-dimensional imaging of the whole process, including the preischemic control phase.

Several of the determined parameters served equally as markers for the differentiation between malignant and benign courses. All cats included in the malignant group exhibited eye dilatation and subsequent transtentorial herniation, whereas cats with benign course did not. In the malignant group, significant and drastic alterations of ICP, CPP, glutamate, and GABA were observed throughout the course of the reperfusion period after primary recovery, whereas in the benign group, sustained recovery of all parameters was typical.

Given a fixed duration, the degree of blood flow reduction during the ischemic episode and therefore the severity of ischemia must be considered most influential for further outcome. Sequential PET determinations of ischemic CBF in an animal that exhibited a malignant course revealed not only the progressive nature of this process but also that almost the entire MCA territory was involved. This is presumably typical for cases with poor outcome, but comparative PET or MRI studies would need to confirm the assumption that volumes of the ischemic territory differ between malignant and benign groups. LDF measurements revealed only minor differences, with a tendency toward lower CBF values in the

group with a malignant course. Spontaneous partial CBF recovery may also have played a role for group differentiation, but CBF measurements by LDF do not provide a means to reliably quantify CBF in the low range. Furthermore, some spontaneous CBF recovery was seen in both groups during the occlusion (Figure 1). In contrast, glutamate elevation during MCAO may serve as a good indicator for group differentiation. It was much more pronounced and peaked later during occlusion in the group with a malignant course compared with the group with a benign course. This result may document best that ischemia was more severe in the malignant group and corresponds to results obtained by Matsumoto et al[22] in a rabbit model of focal ischemia. In this study the amount of ischemic glutamate elevation was positively correlated with the secondary elevation of glutamate after reperfusion.

Excitatory amino acids are considered to play an important role in the evolution of ischemic damage. Extracellular glutamate in particular is known to cause brain damage in vitro.[14] In experimental stroke, the volume of ischemic damage and the magnitude of amino acid release are correlated.[17] Glutamate starts to rise at blood flow thresholds of approximately 20 mL/100 g per minute and may therefore play a role in the progressive destruction of the ischemic penumbra.[18,20,43] In our study, therefore, glutamate elevation during ischemia should be considered not only a reliable predictor of secondary deterioration but also an important cause for such a destructive process. Through a cascade of mechanisms, glutamate elevation may lead to infarct enlargement and further enhancement of glutamate efflux through positively controlled feedback mechanisms. The distinction between malignant and benign course possibly will depend on rather small differences in the primary degree and volume of ischemia immediately following arterial occlusion.

In addition to elevations during the ischemic phase, some reports have documented a secondary rise of glutamate after reperfusion following prolonged transient focal ischemia in cats and rabbits.[21–23] In these reports, both a secondary decline of blood flow and perturbations of transmitter release or reuptake systems due to reperfusion injury have been discussed.[22] Studies that have shown glutamate antagonists to be neuroprotective if applied after reperfusion support the idea of a role of excitotoxins for secondary deterioration in the reperfusion phase.[44,45] In the aforementioned studies, ICP and CPP have not been assessed. Our study shows that after prolonged ischemia of 3 hours, ischemic levels of glutamate recovered rather soon after reperfusion. Glutamate increased again only in the malignant group and only when CBF decreased below a threshold level of approximately 20 mL/100 g per minute. The time point of this glutamate rise coincided with a drop in MABP that was presumably caused by medullary disturbance in the terminal stage of herniation. Secondary glutamate elevation occurred only if symptoms of herniation such as Cushing's phenomenon or pupil dilatation were apparent. The dramatic elevation of ICP and the concomitant decrease of CPP in the later course of reperfusion, with subsequent drop of MABP, most probably pushed CBF below ischemic thresholds for glutamate elevation. We have recently reported that in an epidural balloon expansion model, cortical glutamate increased when eye dilatation and transtentorial herniation were apparent. At this stage, CPP had dropped to approximately 40 to 50 mm Hg.[46] The CPP threshold for glutamate elevation found in the balloon model was similar to that found in the present study in the malignant group.

LDF measurements of CBF provided a less clear threshold for glutamate elevation, presumably because LDF measurements after recirculation varied as a result of movements of the swelling brain in relation to the fixed LDF probe. Sequential PET measurements revealed, however, that a secondary drastic CBF decrease to <20 mL/100 g per minute started when CPP fell to <50 to 60 mm Hg. This coincided when symptoms of herniation were apparent. Subsequent global supratentorial ischemia seen in PET images may also explain that in the present study, histological evaluation disclosed not only large infarcted regions in the territory of the occluded MCA but also early neuronal necrosis in cortical regions contralateral to the primary ischemic focus. This damage can only be explained by critical global CBF reduction in the final stage of herniation.

The question arises as to whether secondary glutamate elevation is of any significance for further deterioration in the course of malignant edema formation after reperfusion. In the model investigated in this study, glutamate rose only after CPP had reached very critical levels below approximately 50 mm Hg. At this late stage, treatment directed against glutamate toxicity seems quite irrelevant. Attempts to avoid the progression into a malignant course should rather be targeted directly against edema formation and brain swelling when it is considered that their evolution occurs at much earlier time points. Management of ICP, including decompressive surgery, may be an alternative method of choice.[47] It should be considered, however, that the course of severe human stroke might differ in several aspects from our cat model. First, the cat ischemia/reperfusion model encompasses a sudden onset of recirculation after removal of the arterial occluder, whereas recanalization through either spontaneous or recombinant tissue plasminogen activator–induced thrombolysis in humans is a comparatively slow process.[48] Second, the rapid, progressive edema formation and mass lesion in cats may not easily be comparable to the time course of deterioration in human stroke patients. One important difference is perhaps that cats possess a bony tentorium that may favor a faster progression into herniation than we would expect in other species and also in human patients.

As specified above, various parameters monitored in the course of ischemia and reperfusion provide good correlates and are therefore good markers of a malignant course. Besides the elevation of glutamate and other extracellular substrates during ischemia, the increase of ICP and decrease of CPP along with the delayed, secondary glutamate rise during reperfusion have such marker function. In view of the critical condition that is generated by malignant edema formation, the more important question is, however, whether monitored variables have a prognostic value to predict the malignant course as early as possible. For example, it has been shown in a rat model that decompressive surgery is most effective if started early after reperfusion.[49] When one con-

siders the temporal patterns of the various observed parameters, such early predictor function is provided best by the pronounced glutamate rise during ischemia, followed by the fatal rise of ICP and the concomitant drop of CPP after reperfusion. In that respect, signs of herniation and eye dilatation as well as secondary elevation of glutamate or other substrates monitored by microdialysis would be less relevant because they appear at a late stage. Under clinical conditions, early monitoring with microdialysis is difficult because several criteria, such as a clear indication by CT or the agreement of patients or of relatives, are prerequisites for the timely application of this invasive technique. The decision on the site of microdialysis probe implantation in patients also seems problematic. In the few microdialysis studies in stroke patients performed thus far, remote locations have been chosen. An original report on a case with fatal stroke showed increased glutamate and glycerin and lactate/pyruvate ratio levels in the contralateral hemisphere 24 hours before ICP rise and subsequent brain death.[24] In a more recent study of 10 patients that applied microdialysis in the immediate vicinity of the infarct, results were more variable, and the time lag between substrate elevation and appearance of herniation was rather short or did not exist.[25] Considering the results of our study, we think that microdialysis probe locations outside the ischemic territory presumably do not provide predictive patterns at early time points. Early prediction might be achieved if probes can be located within ischemic foci. Such locations would have the additional advantage that traumatization of remote regions by implantation of the probes could be avoided.

As evident from our supplementary PET experiments, modern imaging techniques would presumably provide best requirements for early prediction of fatal stroke. Attempts have been successfully undertaken by applying diffusion-weighted MRI,[50] early cerebral CT scanning for assessment of attenuated corticomedullary contrast,[51] and flumazenil PET.[52] Timely application of neuroimaging techniques is often difficult, and sequential measurements are not readily performed in the clinic. Thus, a combination with invasive monitoring techniques such as microdialysis or ICP measurement seems favorable, particularly if implantation of invasive techniques is guided by early neuroimaging.

Acknowledgments

This work was supported by a grant of the Bundesministerium für Bildung und Forschung (Kompetenznetz Schlaganfall) to Drs Graf and Heiss. We were very fortunate to have had the excellent technical assistance of Paula Gabel, Christiane Kleineberg, Andreas Beyrau, and Doris Lattacz. We would also like to thank our colleagues Dr C. Dohmen and Dr S. Schneweis for many fruitful discussions.

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